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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2004905501 for a patent by THE AUSTRALIAN NATIONAL UNIVERSITY as filed on 23 September 2004.

WITNESS my hand this
Nineteenth day of November 2004

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Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Methods of dispersion"

The invention is described in the following statement:

METHODS OF DISPERSION

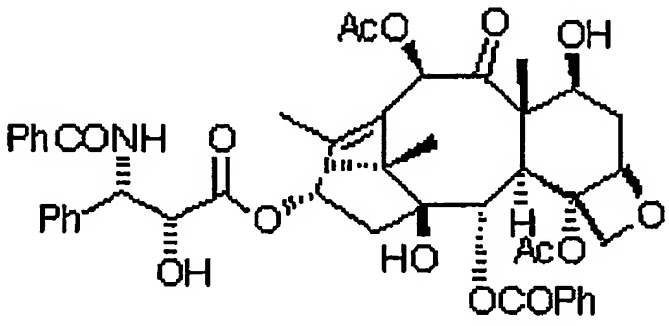
Field of the Invention

The present invention relates to methods of dispersing hydrophobic pharmaceutically active agents in an aqueous phase and to dispersions obtained thereby. Advantageously, methods of the invention circumvent the need for additional surfactants, stabilizers or dispersants. The resulting dispersions may provide new and effective drug delivery systems.

Background to the Invention

Many drugs are derived from natural products and generally have a high degree of complexity in their structure; because of this they are usually water-insoluble oils or solids, lacking the necessary polar nature to dissolve in water. This is a major problem for industry, as many drugs cannot be taken past the testing phase of approval, as suitable aqueous-based drug delivery systems cannot be easily formulated (Bodor, Chemical Aspects of Drug Delivery Systems; Karsa, D. R., Stephenson, R. A., Eds; Royal Society of Chemistry: London, 1996). For the drugs where suitable solvent systems can be found this is usually achieved by placing the drug in a highly insoluble oil, which is then partially dispersed in water either with the aid of a chemical surfactant or meta-stably dispersed with the aid of physical agitation. A major side-effect is that upon introduction to the blood stream the oils used are often harmful to the body in high quantities or must be used quickly to achieve the dose required before the meta-stable dispersion breaks down. However the most harmful side effect is the presence of possible surfactant degradation products from the oils (or the chemical stabilizing surfactants) that can pose their own problems such as the hemolytic cleavage of cells (Davis, *Interdisciplinary Science Reviews 25 (3)*: 175-183, 2000). The ability to disperse hydrophobic oils or the drug directly in water is therefore very beneficial to the industry.

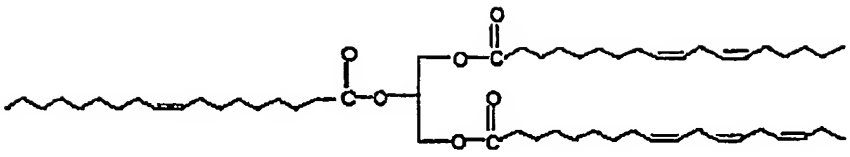
One example is the highly insoluble anticancer drug, paciltaxel (Taxol). The lack of solubility in water is evident from the complex, mostly hydrocarbon structure shown below:



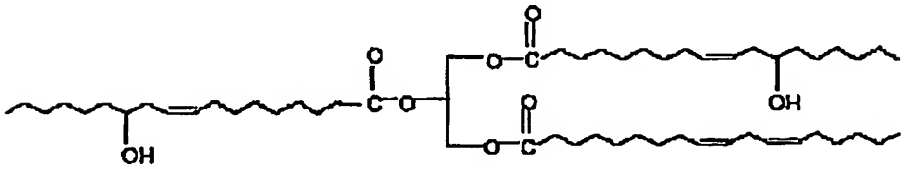
Taxol (Anti-cancer drug)

Taxol is soluble in soybean oil, which can then be dispersed in water with the aid of surfactants to stabilize the emulsion. The drugs used to treat cancer are often highly insoluble in water and as such current delivery systems involve either dispersing the drug into an appropriate drug delivery oil and then dispersing this into water or dispersing the drug directly into water and then injecting it intravenously, although the former is far more prevalent (Stuchlik, *et al.*, *Biomed. papers 145 (2)*: 17-26, 2001). The drug delivery oils used, such as soybean oil, are often unstable and hydrolyze causing harmful side effects in patients such as hemolytic cleavage of blood cells. Even low concentrations (2%) surface-active molecules in the total volume of the drug delivery oil can cause significant health problems (Spiteller, *Medical Hypothese 60 (1)*: 69-83, 2003).

The current oils used for intravenous drug delivery are mainly derived from natural products including rapeseed and cottonseed oil, however the two most commonly used are soybean oil and castor oil (Stuchlik, *et al.*, *Biomed. papers 145 (2)*: 17-26, 2001). The structures of these two oils are shown below:



Triglyceride (Soybean oil)



Castor Oil

These two oils are used because they are hydrophobic, hence water-insoluble drugs will usually dissolve into them. These oils are currently used in industry, and it may well be that, the surfactant by-product produced by hydrolytic cleavage of the tri-ester linkage aids in the dispersion of the oil into the aqueous phase. However this beneficial side-product (the very thing aiding the process) is largely responsible for the harmful side effects and as such the industry monitors the purity of the oils carefully.

There exists, therefore, a need for methods for preparing pharmaceutically acceptable compositions comprising a hydrophobic pharmaceutically active agent, such as a hydrophobic drug, in an aqueous phase, without the substantial use of additional stabilizers, surfactants or dispersants.

Summary of the Invention

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

The natural hydrophobicity of many drugs makes it very difficult to use them for water-based intravenous injection. This lack of water solubility also hinders the development and testing of new drugs. Clinical tests are often refused if the drug can only be dissolved in water-insoluble oils and therefore cannot be administered safely or easily. It has been discovered that de-gassing a mixture of a pharmaceutically acceptable hydrophobic oil carrier or hydrophobic drug and water produces, on vigorous shaking, a uniform fine dispersion of oil droplets, which are of suitable size for intravenous injection. These dispersions are stable and yet do not require the use of added stabilizing agents, such as surfactants and polymers, which can lead to harmful side effects. These dispersions may offer safer drug delivery systems and also might be used in facilitating the development or testing of new experimental, water-insoluble drugs. This novel process has been used to enhance the dispersion of the commonly used drug delivery oils, soybean oil and perfluorooctyl bromide (PFOB). This process can also be applied to other drug delivery oils, which are immiscible with water. For example, the dispersion of perfluorohexane in water is greatly improved by de-gassing. Over time, the dispersions phase separate but are easily re-generated simply by shaking, when stored under de-gassed conditions in sealed vials. The process has also been successfully applied to the hydrophobic drug Propofol, where dispersion was obtained without the use of carrier oil or added dispersants.

The energy required to deform an oil droplet in water, through collisions or shear forces, depends on its size and its interfacial tension. The interfacial tension of hydrophobic droplets dispersed by the de-gassing process will typically be in the range 30-50 mJm^{-2}. By comparison, a typical emulsion droplet, stabilized by added surfactants, has an interfacial tension of about 0.1 mJm^{-2}. The deformation energy required for the same size droplet will depend directly on the interfacial energy. Hence, de-gassed dispersions will have droplets with 300-500x higher surface tension, and hence rigidity compared with emulsion droplets. Hence drugs can be delivered in rigid sub-micron droplets eg finer syringes/ aerosols than normal emulsions. In the blood stream the rigid spheres will also have a higher chemical potential of about 3x initially, due to the higher Laplace pressure and the thermodynamic relation: $\partial\mu = \Delta P * V$. However, this will fall and the particles will become more fluid as lipids in the blood adsorb to the droplet surface and reduce the tension, probably facilitating drug release in the process. Hence, de-gassing should make the delivery process more robust.

Accordingly, the present invention provides a method for preparing a dispersion of a hydrophobic pharmaceutically active agent in an aqueous phase comprising:

a) combining said agent and aqueous phase to form a mixture; and
b) before, during or after said combining, removing dissolved gases from one or both of the active agent and aqueous phase.

Optionally, the active agent may first be dissolved or dispersed in a suitable pharmaceutically acceptable hydrophobic carrier oil or liquid.

In a preferred embodiment, the invention provides a method for dispersing a hydrophobic pharmaceutically active agent in an aqueous phase comprising:

a) combining said agent and aqueous phase to form a mixture; and
b) removing dissolved gases from said mixture.

The process of removing the gas from a mixture of the agent and aqueous phase, may result in spontaneous dispersion of the agent in the aquesous phase. Alternatively, the dispersion may be generated, or regenerated after settling, by agitating or shaking the mixture, still under vacuum.

Thus, in a further embodiment, the method comprises the additional step of :

c) agitating or shaking the degassed mixture to form a dispersion.

The invention also provides a dispersion of a hydrophobic pharmaceutically active agent in an aqueous phase, substantially free of additional stabilizers, surfactants and dispersants. In particular, the invention provides a dispersion substantially free of dissolved gases or a dispersion wherein the agent or agent+carrier and/or aqueous phase are substantially free of dissolved gases.

In a preferred embodiment, the invention provides a drug delivery system comprising a hydrophobic pharmaceutically active agent in an aqueous phase, said drug delivery system substantially free of additional stabilizers, surfactants and dispersants. In another preferred form, the drug delivery system is substantially free of a carrier.

Yet another aspect of the invention relates to a dispersion or drug delivery system obtainable by the methods described herein.

The invention also provides a dispersion of droplets of a hydrophobic pharmaceutically active agent in an aqueous phase wherein the droplets have an interfacial tension of about 30-50 mJm^{-2}

Description of the Invention

As used herein "hydrophobic pharmaceutically active agent" is intended to include any hydrophobic or water immiscible physiologically active drug which elicits a physiological response in a subject upon administration. The drug may be liquid , oil or solid. Examples of classes of hydrophobic drugs contemplated by the invention include analgesics and anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, anti-coagulants, anti-bacterial agents, anti-depressants, anti-diabetics, anti-epileptics, anti-fungal, anti-muscarinic agents, anti-neoplastic agents and immunosuppressant, anti-protazoal agents, anti-thyroid agents, anxiolytic, sedatives, hypnotics and neuroleptics, Ǝ-Blockers, cardiac inotropic agents, corticosteroids, diruetics, anti-parkinsonian agents, gastro-intestinal agents, histamine H_1-receptor antagonists, lipid regulating agents, nitrates and other anti-anginal agents, nutritional agents, opioid analgesics, sex hormones and stimulants. Some examples thereof include:

Analgesics and anti-inflammatory agents: aloxiprin, auranofin, azapropazone, benorylate, diflunisal, etodolac, fenbufen, fenoprofen calcim, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamic acid, mefenamic acid, nabumetone, naproxen, oxyphenbutazone, phenylbutazone, piroxicam, sulindac.

Anthelmintics: albendazole, bephenium hydroxynaphthoate, cambendazole, dichlorophen, ivermectin, mebendazole, oxamniquine, oxfendazole, oxantel embonate, praziquantel, pyrantel embonate, thiabendazole.

Anti-arrhythmic agents: amiodarone, disopyramide, flecainide acetate, quinidine sulphate.

Anti-bacterial agents: benethamine penicillin, cinoxacin, ciprofloxacin, clarithromycin, clofazimine, cloxacillin, demeclocycline, doxycycline, erythromycin, ethionamide, griseofulvin, imipenem, nalidixic acid, nitrofurantoin, rifampicin, spiramycin,

sulphabenzamide, sulphadoxine, sulphamerazine, sulphacetamide, sulphadiazine, sulphafurazole, sulphamethoxazole, sulphapyridine, tetracycline, trimethoprim.

Anti-coagulants: dicoumarol, dipyridamole, nicoumalone, phenindione.

Anti-depressants: amoxapine, maprotiline, mianserin, nortriptyline, trazodone, trimipramine maleate.

Anti-diabetics: acetohexamide, chlorpropamide, glibenclamide, gliclazide, glipizide, tolazamide, tolbutamide.

Anti-epileptics: beclamide, carbamazepine, clonazepam, ethotoin, methoin, methsuximide, methylphenobarbitone, oxcarbazepine, paramethadione, phenacemide, phenobarbitone, phenytoin, phensuximide, primidone, sulthiame, valproic acid.

Anti-fungal agents: amphotericin, butoconazole nitrate, clotrimazole, econazole nitrate, fluconazole, flucytosine, griseofulvin, itraconazole, ketoconazole, miconazole, natamycin, nystatin, sulconazole nitrate, terbinafine, terconazole, tioconazole, undecenoic acid.

Anti-gout agents: allopurinol, probenecid, sulphin-pyrazone.

Anti-hypertensive agents: amlodipine, benidipine, darodipine, dilitazem, diazoxide, felodipine, guanabenz acetate, isradipine, minoxidil, nicardipine, nifedipine, nimodipine, phenoxybenzamine, prazosin, reserpine, terazosin.

Anti-malarials: amodiaquine, chloroquine, chlorproguanil, halofantrine, mefloquine, proguanil, pyrimethamine, quinine sulphate.

Anti-migraine agents: dihydroergotamine mesylate, ergotamine tartrate, methysergide maleate, pizotifen maleate, sumatriptan succinate.

Anti-muscarinic agents: atropine, benzhexol, biperiden, ethopropazine, hyoscyamine, mepenzolate bromide, oxyphencylcimine, tropicamide.

Anti-neoplastic agents and Immunosuppressants: aminoglutethimide, amsacrine, azathioprine, busulphan, chlorambucil, cyclosporin, dacarbazine, estramustine, etoposide, lomustine, melphalan, mercaptopurine, methotrexate, mitomycin, mitotane, mitozantrone, procarbazine, tamoxifen citrate, taxol, testolactone.

Anti-protazoal agents: benznidazole, clioquinol, decoquinate, diiodohydroxyquinoline, diloxanide furoate, dinitolmide, furzolidone, metronidazole, nimorazole, nitrofurazone, ornidazole, tinidazole.

Anti-thyroid agents: carbimazole, propylthiouracil.

Anxiolytic, sedatives, hypnotics and neuroleptics: alprazolam, amylobarbitone, barbitone, bentazepam, bromazepam, bromperidol, brotizolam, butobarbitone, carbromal, chlordiazepoxide, chlormethiazole, chlorpromazine, clobazam, clotiazepam, clozapine, diazepam, droperidol, ethinamate, flunanisone, flunitrazepam, fluopromazine, flupenthixol decanoate, fluphenazine decanoate, flurazepam, haloperidol, lorazepam, lormetazepam, medazepam, meprobamate, methaqualone, midazolam, nitrazepam, oxazepam, pentobarbitone, perphenazine pimozide, prochlorperazine, propofol, sulpiride, temazepam, thioridazine, triazolam, zopiclone.

∃-Blockers: acebutolol, alprenolol, atenolol, labetalol, metoprolol, nadolol, oxprenolol, pindolol, propranolol.

Cardiac Inotropic agents: amrinone, digitoxin, digoxin, enoximone, lanatoside C, medigoxin.

Corticosteroids: beclomethasone, betamethasone, budesonide, cortisone acetate, desoxymethasone, dexamethasone, fludrocortisone acetate, flunisolide, flucortolone, fluticasone propionate, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone.

Diuretics: acetazolamide, amiloride, bendrofluazide, bumetanide, chlorothiazide, chlorthalidone, ethacrynic acid, frusemide, metolazone, spironolactone, triamterene.

Anti-parkinsonian agents: bromocriptine mesylate, lysuride maleate.

Gastro-intestinal agents: bisacodyl, cimetidine, cisapride, diphenoxylate, domperidone, famotidine, loperamide, mesalazine, nizatidine, omeprazole, ondansetron, ranitidine, sulphasalazine.

Histamine H_1-Receptor Antagonists: acrivastine, astemizole, cinnarizine, cyclizine, cyproheptadine, dimenhydrinate, flunarizine, loratadine, meclozine, oxatomide, terfenadine.

Lipid regulating agents: bezafibrate, clofibrate, fenofibrate, gemfibrozil, probucol.

Nitrates and other anti-anginal agents: amyl nitrate, glyceryl trinitrate, isosorbide dinitrate, isosorbide mononitrate, pentaerythritol tetranitrate.

Nutritional agents: betacarotene, vitamin A, vitamin B_2, vitamin D, vitamin E, vitamin K.

Opioid analgesics: codeine, dextropropyoxyphene, diamorphine, dihydrocodeine, meptazinol, methadone, morphine, nalbuphine, pentazocine.

Sex hormones: clomiphene citrate, danazol, ethinyl estradiol, medroxyprogesterone acetate, mestranol, methyltestosterone, norethisterone, norgestrel, estradiol, conjugated oestrogens, progesterone, stanozolol, stibestrol, testosterone, tibolone.

Stimulants: amphetamine, dexamphetamine, dexfenfluramine, fenfluramine, mazindol.

Under certain circumstances it may be advantageous or desirable to incorporate (dissolve or disperse) the drug and/or one or more other agents into a pharmaceutically acceptable hydrophobic carrier and disperse this mixture in the aqueous phase. Thus, the drug, either a liquid or a solid, may be dispersed directly into the aqueous phase or dispersed or dissolved in a hydrophobic carrier liquid or oil before dispersion into the aqueous phase. Suitable hydrophobic carriers include those physiologically inert or pharmaceutically acceptable carriers which have a droplet contact angle of at least about 80°, preferably at least about 90° as described below, or alternatively are hydrocarbons of greater than 8 carbon atoms. Alternatively, carriers with a water solubility of less than about 0.1 %, preferably less than 0.01% may be suitable.

The degassing oil/water dispersion process is more effective with insoluble carrier liquids or oils rather then the partially soluble ones. This can be attributed to the fact that the more soluble oils undergo Ostwald ripening, allowing rapid oil droplet growth. The more hydrophobic an oil the better it is for this process as Ostwald ripening cannot occur. A degree of hydrophobicity can be estimated by applying the Young's wetting equation to a theoretical liquid/liquid drop profile. A theoretical water droplet contact angle on the oil surface can be calculated, and if this angle is higher than about 80°, preferably higher than 90° then the oil is sufficiently hydrophobic. For example, for dodecane, the droplet contact angle is 110 °.The following table shows the calculated water contact angles and we can see that these natural oils both have a degree of hydrophilicity that in tandem with the presence of surfactants from degradation will aid the dispersion. However the more hydrophobic of the two drug delivery

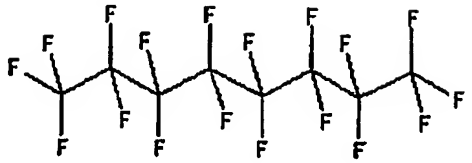
oils is soybean oil, as it has the higher theoretical water contact angle. The effect of de-gassing on the dispersion of this oil was studied.

Physical properties:

	Castor oil	Soybean oil
Interfacial tension (mN/m)	3.5	15.0
Surface tension (mN/m)	39.0	25.0
Density (g/ml)	0.96	0.916
Theoretical contact angle	60.5^0	82.0^0

It was discovered (see later results) that for soybean oil the calculated (equilibrium) water droplet contact angle does not properly reflect its potential for enhanced dispersion on de-gassing. This is because rapid dispersion, on vigorous shaking, does not allow the interface time to stabilize. Thus, the large amphiphilic soybean molecule cannot readily orientate itself as a new water-oil interface is rapidly created. Thus the oil behaves more like a liquid hydrocarbon, with a correspondingly high, transient, interfacial tension. It is for this reason that de-gassing has a strong effect on soybean oil dispersion.

Perfluorocarbons make use of the particular strong C-F bond, which is even stronger when there are several fluorines bonded to a single carbon. These molecules are therefore quite inert which makes them potentially useful as drug delivery oils. A perfluorocarbon is any fluorocarbon where the bulk (say at least 60%) of the non C-C bonds are C-F bonds. Partially fluorinated hydrocarbons are also contemplated by the invention. The structure of a typical linear perfluorocarbon molecule is shown below:



Perfluorooctane C_8F_{18}

Another of the interesting aspects of perfluorocarbons is their high degree of hydrophobicity, which makes them perfect for the degassing process. Perfluorocarbons have a very low surface tension against air, while having a very high interfacial tension against water, this gives them a very high water droplet contact angle. This high water contact angle means that they are very hydrophobic (see following table) making them perfect candidates for the degassing process. Perflubron is the generic name for perfluorooctyl bromide, the perfluorocarbon drug delivery oil used in the industry.

Physical properties:

	C_7F_{16}	C_6F_{14}	C_8F_{18}	Perflubron
Interfacial tension (mN/m)	39.7	38	42	-----
Surface tension (mN/m)	12.85	11.91	14.00	18
Density (g/ml)	1.75	1.669	1.73	1.93
Theoretical contact angle	112^0	111^0	113^0	------

Although perfluorocarbons are currently of significant medical use there is no easy or cheap way to deliver the drug intravenously. Currently, fluorocarbons are dispersed in a similar manner to hydrocarbons using, for example (in the case of perfluorooctyl bromide), a small amount of the fluorocarbon detergent perfluorodecyl bromide as dispersant oil, which is expensive and may be toxic to the kidneys.

Suitable carriers include those commonly used in the art of pharmacy and include soybean oil, castor oil, rapeseed oil, cottonseed oil and perfluorocarbons such as perfluorohexane, perfluoroheptane and perfluorooctane as well as perfluorooctylbromide.

The term "aqueous phase" includes water, or, where appropriate, mixtures of water and a water miscible or soluble solvent or compound. Suitable solvents might include alcohols (eg EtOH, PrOH) and DMSO.

Optionally, the compositions or drug delviery systems of the invention may also comprise one or more additional additives or excipents such as flavourants, colourants, preservatives, buffers, isotonic agents and antioxidants. These may be incorporated into the compostion or drug delviery systems at an appropriate stage, as necessary, dependent on whether they are hydrophobic or hydrophilic, either in the aqueous phase or with a suitable hydrophobic carrier, or after these components have been mixed or degassed. Such excipients or additives are known in the art of pharmacy (see for example, *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing)

The drug to be dispersed in the aqueous phase may be a liquid or a solid at room temperature. Preferably, the solid for dispersion is a finely divided solid of 2μm or less, more preferably 1μm or less and more preferably submicron particulate size, such as less than 0.5μm, preferably about 0.4-0.3μm.

Dispersions obtained by the methods of the invention afford colloidal emulsions which are substantially monodispersed having a droplet size of less than 2 μm, more preferably, 1 μm, preferably about 0.6-0.5μm. Preferably, the resulting dispersions are stable for at least 1 hour, more preferably at least 3-4 hours or up to at least 24 hours. Particularly preferred dispersions may be stable for at least 3-4 days, one week or 3-4 weeks. The dispersions are particularly suitable for use as injectable drug delivery systems as they are not readily subject to shear, and

thereby may circumvent problems associated therewith, such as viscosity increase. The methods of the invention may also advantageously, where desirable, allow for the preparation of drug delivery systems having an increased concentration of the desired drug when compared to know dispersion methods, such as those which utilise the use of additional surfactants, stabilizers or dispersants.

Dependent upon the nature of the agent and any carrier used, the mixture of agent and aqueous phase may spontaneously disperse during degassing. Alternatively, a further optional step in the methods of the invention involves the step of shaking or agitating the degassed mixture to form a dispersion.

The methods of the invention advantageously afford access to compositions suitable for use as drug delivery systems substantially free of stabilizers, surfactants and other dispersants. Such drug delivery systems may be presented for oral, rectal, nasal, topical (including dermal, buccal and sublingual), vaginal or parental (including subcutaneous, intramuscular, intravenous and intradermal) use and may include other components known in the art of pharmacy. Particularly preferred drug delivery systems are for injectable use.

Preferably the dissolved gases are removed from the agent/agent+carrier/aqueous phase (degassed) by the "freeze, pump, thaw" method. Thus, the agent or agent+carrier and aqueous phase, either individually or as a mixture, is frozen in liquid nitrogen and out-gassed by a vacuum pump. Following removal of the gas, the component(s) or mixture are then allowed to thaw and remaining dissolved gases are drawn into the space above the liquid. The "freeze, pump, thaw" cycle may be performed once or more preferably at least 2, 3, 4 or 5 times. Preferably at least 80% of dissolved gas is removed from the system, more preferably at least 90% or 95 %. Most preferably at least 97 or 99% of dissolved gasses are removed and even more preferably at least 99.99%. A dispersion or drug delivery system or component thereof "subtantially free of dissolved gases" refers to a dispersion or system or component thereof

wherein at least 80% of dissolved gas is removed, more preferably at least 90% or 95 %. Most preferably at least 99% of dissolved gasses are removed.

The invention also provides a method of enhancing the dispersion of a hydrophobic pharmaceutically active agent in an aqueous phase. "Enhancing" is intended to refer to the improved dispersion and/or stability of an agent in an aqueous phase wherein one or both or the agent and aqueous phase has been degassed compared to the corresponding non-degassed case.

The enhancement of oil droplet dispersion in water is most easily monitored using turbidity measurements. This enhancement can be measured by the difference between the new system (degassed) and the gassed blank, following vigorous shaking. In general, the gassed dispersion without the aid of stabilizing surfactants, is very unstable and phase separates readily, whereas the degassed mixture is far more stable and can take days to phase separate. Turbidity is a measure of how many droplets are dispersed in a given phase and is measured in NTU (nephelometric turbidity units) and in the results presented here is measured by light scattering. To give an understanding of the magnitude of these turbidity values, distilled water has a turbidity of 0.02 NTU, while tap water has a value of 1-2 NTU. Although useful, NTU measurements are of limited value and the results can be inaccurate if the refractive index of the dispersed phase is close to that of the dispersing phase (such as with perfluorocarbons in water) and so in some cases dynamic light scattering (DLS) has been used to obtain the droplet size distribution, as well as the charge on the oil droplets. However, careful interpretation of the DLS results is required for poly-disperse samples. Mono-disperse samples show size distribution by volume graphs (see later) over similar size ranges to the Z-average (diameter) and have a small PDI value (poly-dispersity index). The magnitude of the PDI is a measure of poly-dispersity. For poly-disperse samples the Z-average is the best estimate of average droplet size.

Although degassing enhances dispersion it is not stable indefinitely. The length of time a droplet is stable is obtained by simply balancing Stokes law with the force due to gravity. The balancing of these two forces determines the droplets settling rate, which is dependant on size and density relative to water. Once this is known along with the length of the tube, an approximate time for the duration of stability can be determined. It has been found, however, that the settling rate is only important for the amount of time that the dispersion is stable *once shaken*, since the original mixture and dispersion can be regenerated simply by vigorous shaking after the two phases separate, assuming that the mixture is stored in a sealed vessel under de-gassed conditions. Oil in water mixtures de-gassed in sealed glass tubes have so far been stored for up to 18 months and these still demonstrate enhanced dispersion on shaking. Once shaken and exposed to air, the dispersed droplets may remain dispersed for up to at least 24 hours, even though gas slowly then diffuses into the mixture. Thus, the dispersions or drug delivery systems of the invention are advantageous in that they may be stored for weeks or even months, and can be redispersed by shaking or agitation and releasing the vacuum (breaking open the sealed ampoule) prior to administration to the patient.

The degassed dispersion was found to contain a mono-disperse droplet size distribution for insoluble hydrocarbon oils . This mono-dispersity can be attributed to the following factors: very small droplets have high velocities (from their kinetic energy) and as such have a higher tendency to collide and coalesce with other droplets. Larger droplets will settle out due to gravitational effects, as was mentioned in the previous section. This leaves a certain size of particle that is not fast enough to overcome the electrostatic repulsion and is too small to settle out quickly. The particles are stable and do not coalesce due to the fact that they are charged and cannot easily be forced together because of an electrostatic repulsion. It has been shown that even when high levels of salt (even above 0.5M) are added to the dispersion, once already formed, the oil droplets do not coalesce.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within the spirit and

scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

The following examples are provided for the purpose of illustrating certain embodiments of the invention and are not intended to limit the generality hereinbefore described.

Examples

Materials and methods

Soybean oil degradation products are surface active, which helps to stabilize the dispersion used for drug delivery. However, these surfactant side products are harmful to human cells and can also, upon agitation, produce a froth that can create its own problems once in the body. Currently before the soybean oil is loaded with a drug it is purified (USP grade) from the soybean. However, as mentioned previously, degradation products do form over time. Storing the soybean oil cold slows the hydrolysis process. If hydrolysis has occurred, it is generally easy to remove the carboxylate surfactant chains via a simple two-phase (solvent/water) separation. This purified version has been used here and compared with the non-purified sample.

Perfluorooctyl bromide, perfluorohexane and Propofol were used as supplied. Water was prepared by activated charcoal and reverse osmosis filtration prior to distillation and storage in Pyrex vessels in a laminar flow filtered air cabinet.

Mixtures of oil and water were de-gassed by a process of repeated freezing in liquid nitrogen, followed by pumping down to a pressure of 0.01mbar and then melting in a sealed tube. The dissolved gas produced on each melting cycle was removed on re-freezing. Although this process was carried out five times, typically no further de-gassing on melting was observed

after 3-4 cycles. The vacuum pressure of 0.01mbar corresponds to a de-gassing level of about 99.999%, if it is assumed that the final pressure achieved on several cycles of freeze/thaw/pumping is given by the pressure in equilibrium with the final frozen liquid, which on being melted does not give any visible bubbling/out-gassing. (It should be noted that membrane separators and vacuum towers are used to de-gas liquids commercially.)

Dispersion of oil in water was achieved by vigorous shaking of the mixture for 8sec in a sealed Pyrex tube. Turbidity was measured using an HF Scientific Micro 100 Turbidimeter. Particle sizes and zeta potentials were measured using a Malvern Zetasizer.

Results

Soybean oil

Degassed, purified soybean oil is substantially better dispersed in de-gassed water, following vigorous shaking, as is shown in Figure 1. Purification also reduces the foaming of the soybean oil/water mixture due to a reduction in surfactant degradation products. As can be seen from the results in Figure 1, the initial dispersion, within say the first minute or so, is substantially enhanced by de-gassing. The enhanced dispersion is maintained for several hours. Figure 2 shows the DLS results on the de-gassed purified and raw samples of soybean oil, 1 hour after vigorous shaking. The purified de-gassed oil gave smaller droplets (of average diameter 3μm), with a narrower range of droplet size variation. Figure 3 shows the droplet sizes for gassed soybean oil mixture 20 mins after vigorous shaking. After 1 hour the signal had no peaks and gave a PDI value of 1.0.

Figure 4 gives the zeta potential of the soybean oil droplets and shows an average value of –60mV for the de-gassed case. For comparison, Figure 5 gives the zeta potential for unpurified, gassed soybean oil, a value of –13mV.

Perfluorooctyl bromide (PFOB)

Figure 6 summarizes the effect of de-gassing on the dispersion of this oil in water. Although the overall turbidity is much lower than for the soybean oil (because fluorocarbon oils have refractive indices close to water), the enhanced dispersion due to de-gassing is clear. The dispersion is maintained for the de-gassed mixture for many hours. The size distribution for PFOB droplets 1 hour after vigorous shaking is shown in Figure 7. The droplets have an average diameter of about 0.6 microns and a fairly narrow size distribution. The corresponding distribution for the gassed case after 1 hour is shown in Figure 8. In this case the droplets are bigger and seem to have a broader size distribution. The zeta potentials of PFOB droplets, for the de-gassed mixture, are shown in Figure 9. The average value was – 42mV.

Perfluorohexane

The effect of degassing on the dispersion of perfluorohexane is summarized in Figure 10. Clearly within less than a minute after vigorous shaking the gassed mixture phase separates into the oil and water, whereas the de-gassed mixture is readily dispersed and maintains its stability for many hours. The difference in turbidity is once again striking because of the similarity in refractive index of the oil (1.29) and water (1.33). A photograph of the difference in turbidity between the de-gassed and the blank, 1-2 min after vigorous shaking, is shown in Figure 11.

Propofol

Propofol is a water insoluble oil, commonly used as a sedative. It can only be delivered intravenously by dissolving in an oil such as soybean and stabilized with added surfactants. Its chemical name is 2,6-diisopropy phenol. The effect of de-gassing on the dispersion of this drug, in the absence of a carrier oil or added dispersants, is clearly demonstrated by the

photograph in Figure 12, which was taken with 1-2 minutes after vigorous shaking. The de-gassed mixture, on the left, shows complete dispersion of the oil, whereas the gassed case has many large, visible droplets of the oil. These results are consistent with those obtained on other hydrophobic liquids. In addition, as with other dispersions, it is most likely that the oil droplets will be of sub-micron size. The dispersion was observed to be stable over many hours, which indicates that the oil droplets must be fine. Clearly, in this case, the drug can be delivered in an aqueous medium without the need for any additives. The turbidity of the de-gassed dispersion was monitored for several hours and the mixture was then exposed to high salt levels, above those found in human blood. The dispersion was unaffected by the addition of salt.

Griseofulvin

The white, finely powdered solid drug griseofulvin was dispersed (0.01g in 25ml) directly into water under both gassed and de-gassed conditions. The solid was clearly well dispersed in the degassed, cloudy solution, whereas solid clumps and a more transparent solution was observed for the gassed solution.

In addition, griseofulvin was dispersed in soybean oil, where 0.05g griseofulvin was dissolved in 10ml of soybean oil, of which 0.2ml of this oil/solid solution was then dispersed in 25ml water. The turbidity results obtained after dispersion, by vigorous shaking, were the same as those obtained for soybean oil alone.

These results demonstrate that solid, hydrophobic drugs can be dispersed directly in de-gassed water and that the addition of drugs into the carrier oil does not affect the de-gassed dispersion.

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FIGURE 1

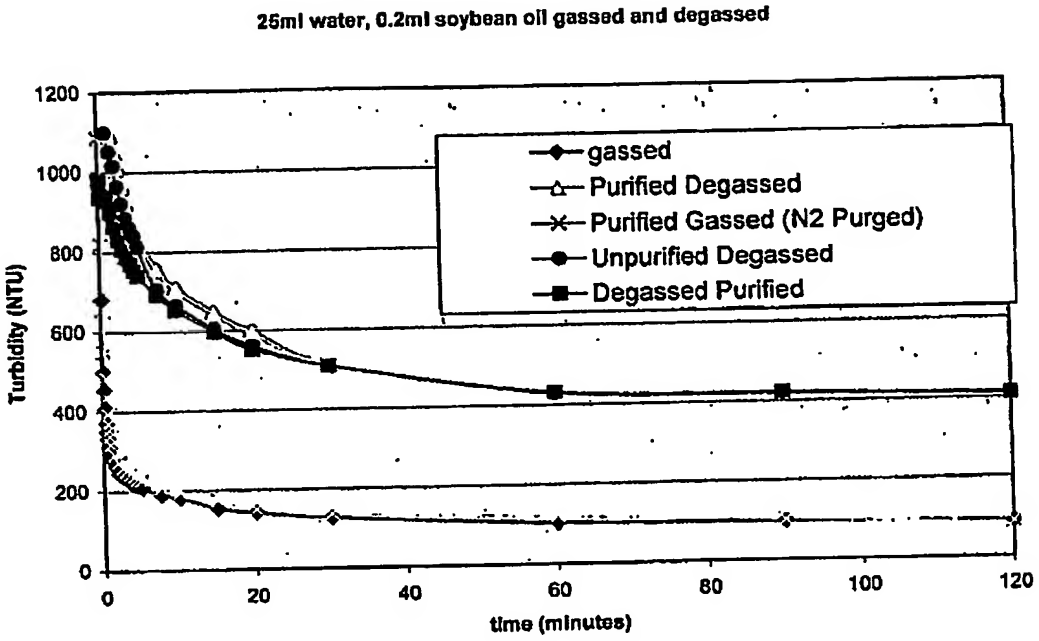
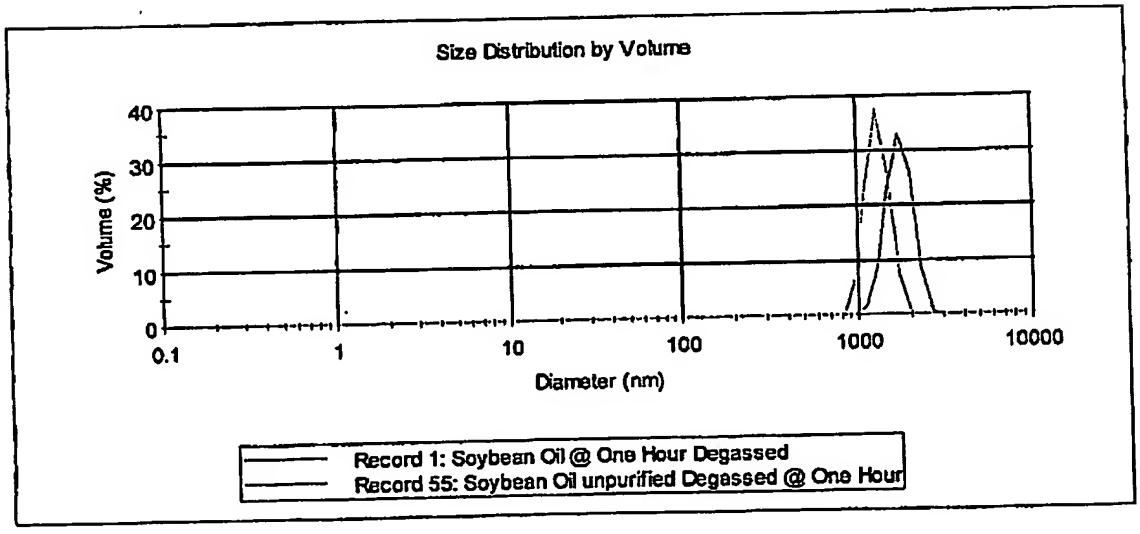
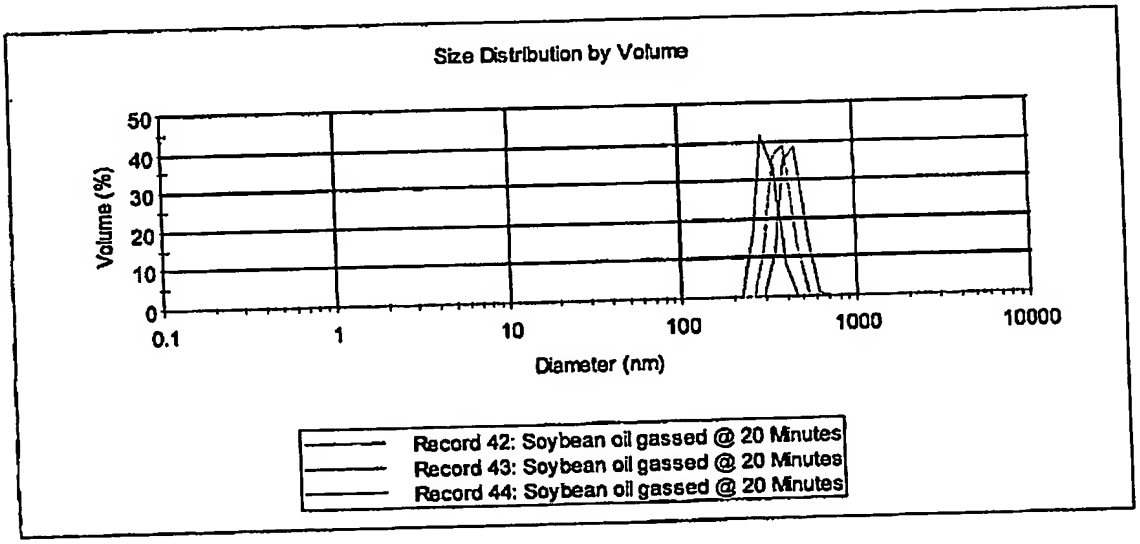


FIGURE 2



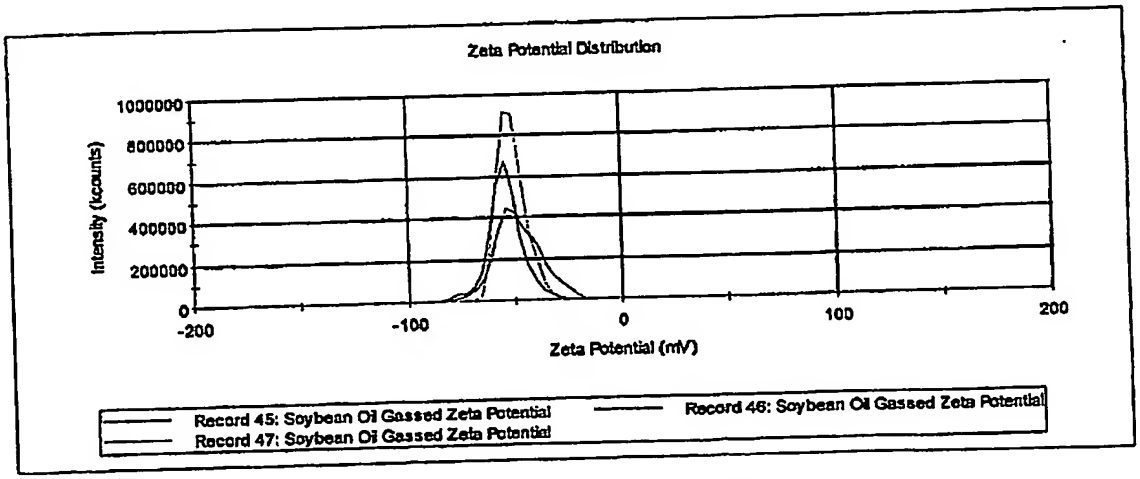
Size graphs for purified (degassed) and unpurified soybean oil (degassed) at one hour (purified = 1696nm and unpurified = 1282nm)
PDI for purified = 0.375 and Z-average size for purified = 3165nm
PDI for unpurified = 0.534 and Z-average size for unpurified = 5811nm

FIGURE 3



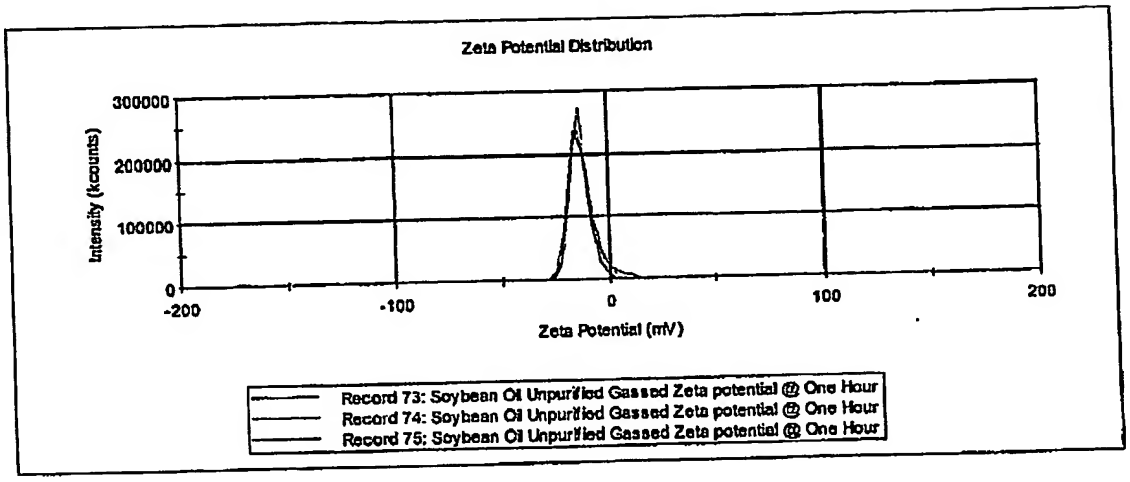
Size Graphs for Soybean Oil (Gassed) at twenty minutes -- the values at one hour were incomprehensible. (Diam = 312.8nm)
PDI = 1.000 and Z-average size = 5477nm

FIGURE 4



Zeta potential graph for soybean oil (degassed) = -59.54mV

FIGURE 5



Zeta Potential Graph for Soybean Oil unpurified (Gassed) = -13.33mV

FIGURE 6

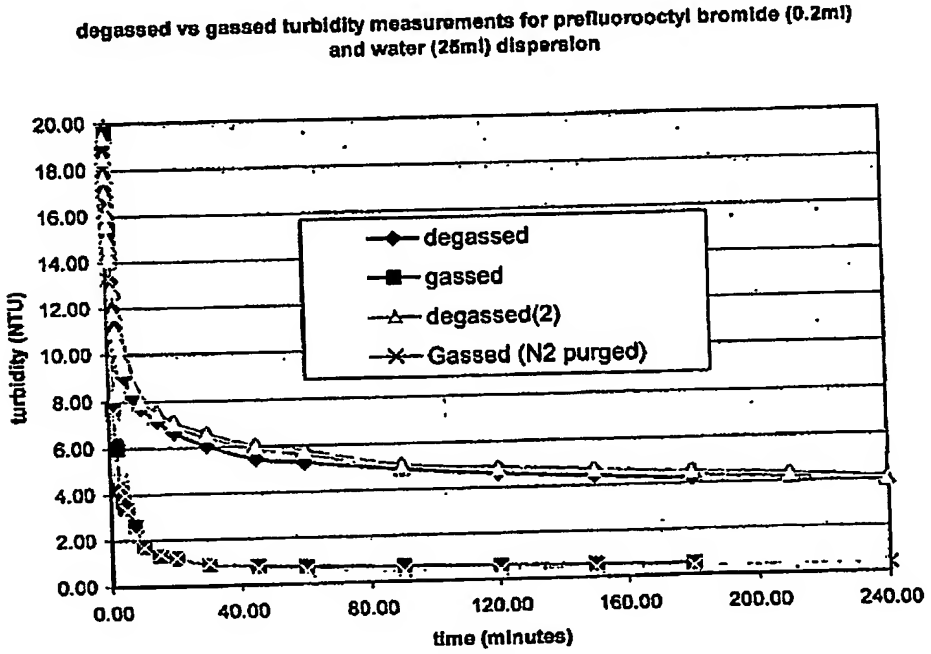
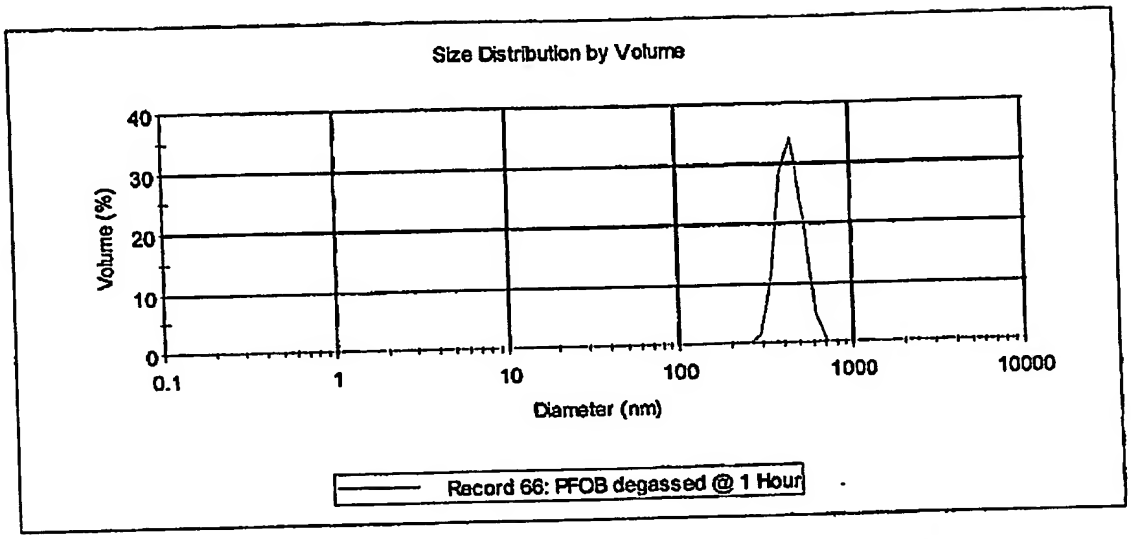
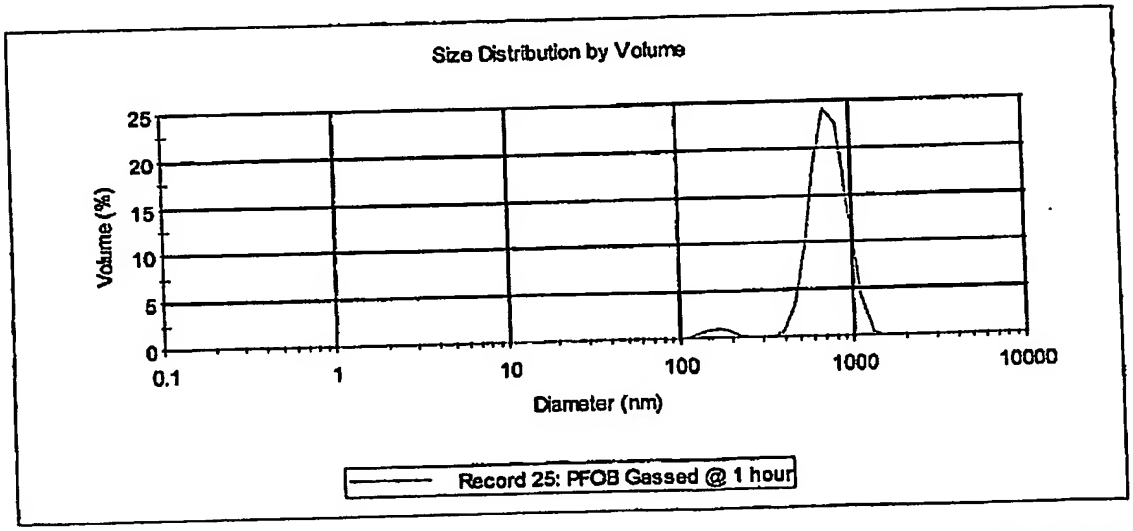


FIGURE 7



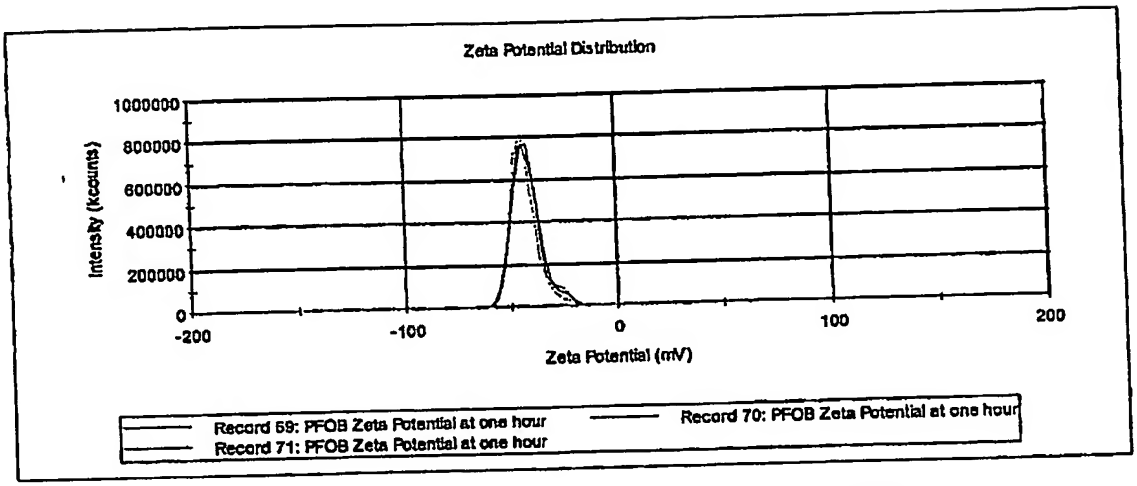
Size Graph for Perflurooctyl Bromide (Degassed) at one hour (Diam 437.6nm)
PDI = 0.430, Z average size = 599.6nm

FIGURE 8



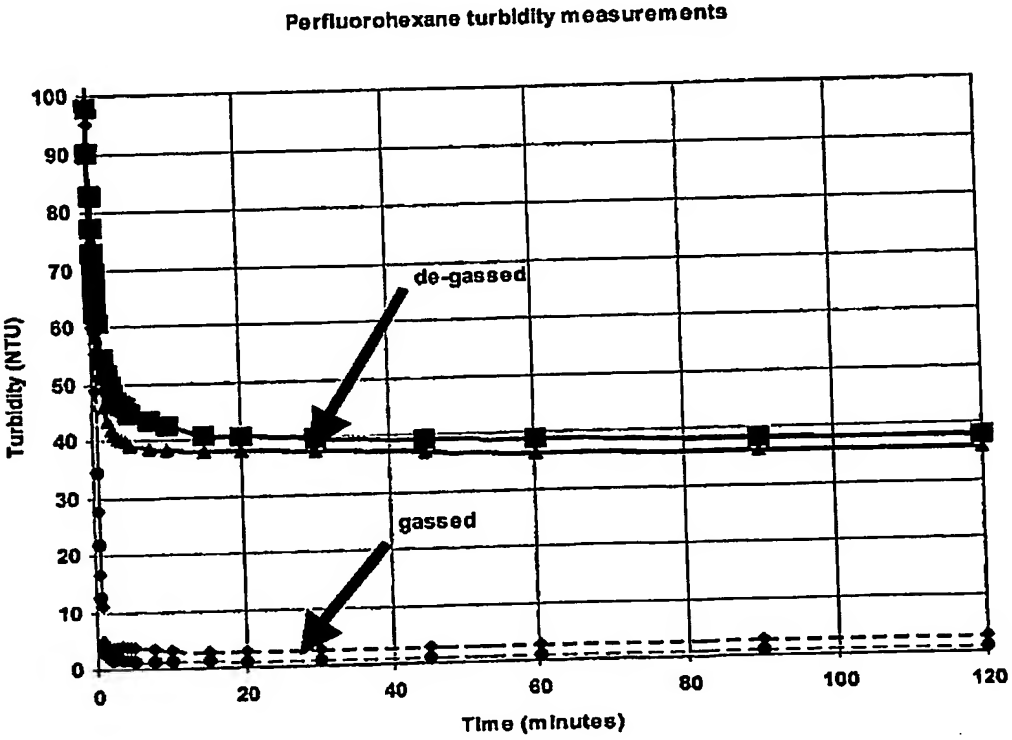
Size graph for Perfluorooctyl Bromide (Gassed) at one hour (Diam 746.1nm and 163.4nm)
PDI = 0.674 and Z-average size = 1176nm

FIGURE 9



Zeta Potential graph for perfluorooctyl bromide (degassed) = -42.13mV

FIGURE 10



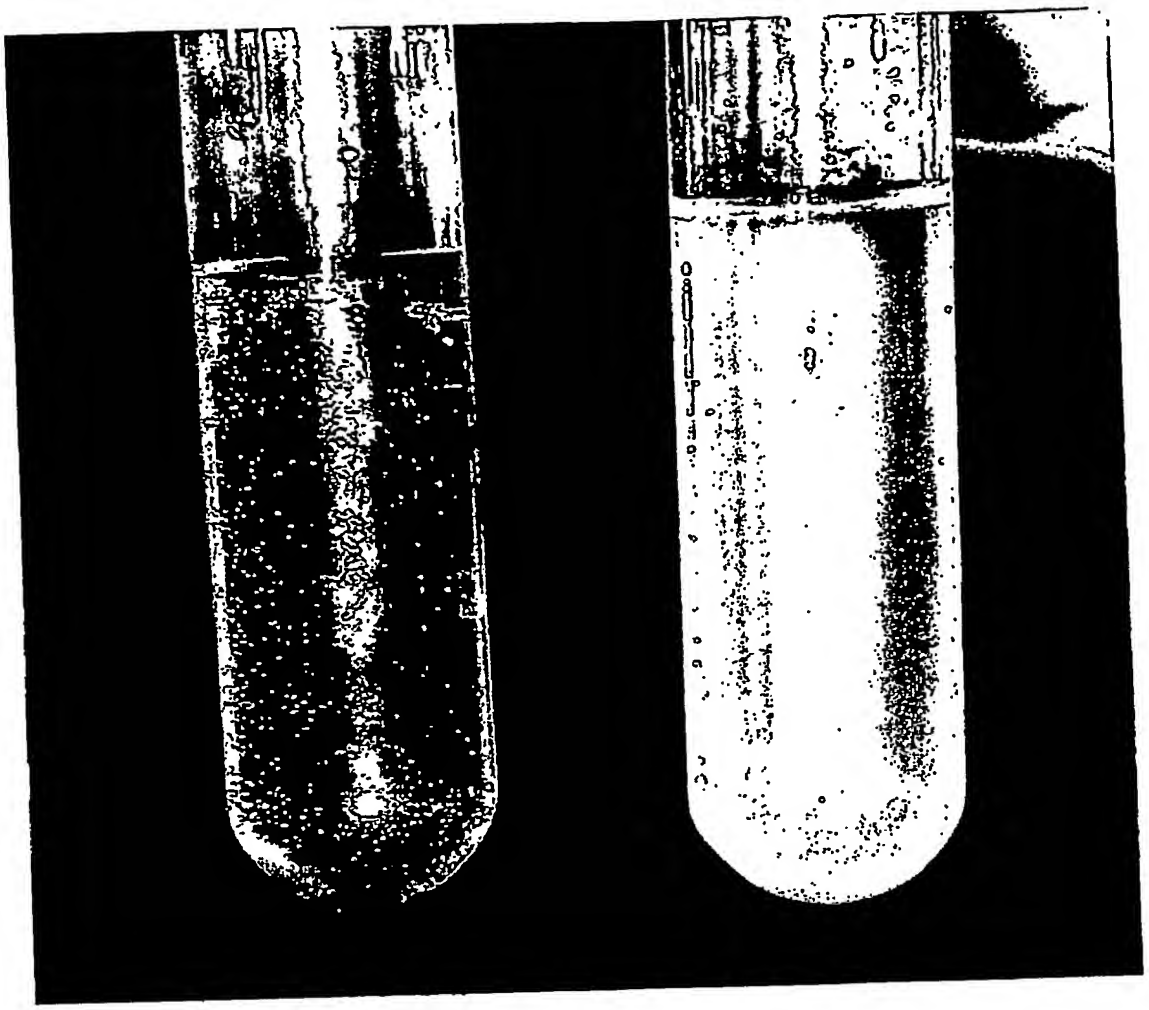


FIGURE 11

FIGURE 12

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